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Peptide-Elicited Protein-Reactive Antibodies in Molecular Biology and Medicine

T. M. SHINNICK, J. G. SUTCLIFFE, N. GREEN, AND R. A. LERNER

Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California, U.S.A.

A relatively short peptide can be used as an immunogen to elicit antibodies that are capable of reacting with full-length proteins containing the peptide's amino acid sequence. Such peptides are frequently represented in the primary sequence of a protein. The antibodies elicited by these peptides are directed against a specific region of the protein chosen in advance by the investigator and so have a predetermined specificity. In basic research, such antibodies are useful in identifying the gene product of an open reading frame, purifying enzymatically active proteins by immunoaffinity techniques, and investigating protein folding domains and immunogenic structure and function. In medicine, since some peptides are capable of eliciting antisera that can bind to and neutralize virus, the antibodies may be reagents for passive vaccination, antitoxin therapy, and targeted immunotherapy of neoplasia. The peptides themselves may serve as the basis for safe, chemically defined vaccines.

The observation that a short oligopeptide corresponding to virtually any surface-accessible region of a protein can elicit antibodies that react with the full-length protein has led to the development of a powerful new technology with applications in both basic research and medicine (reviewed in [1–3]). In part, the utility of this approach lies in the fact that essentially all one needs to produce antibodies that react with a given protein is its amino acid sequence, thus allowing one to circumvent problems often associated with proteins synthesized in small amounts, proteins that are difficult to isolate or purify, proteins associated with known pathogens, or proteins whose existence is merely inferred from a nucleotide sequence. The general approach and the immunogenicity and antigenicity of synthetic peptides have been recently reviewed [1–3]. Here we will briefly review the uses of this approach in basic research and then illustrate some of its possible practical applications in medicine with a review of work on the hepatitis B virus.

APPLICATIONS IN MOLECULAR BIOLOGY

Many of the uses of the protein-reactive peptide antibodies in basic research are reflected in two facets of the approach. First, as mentioned earlier, if one has the amino acid sequence of a protein, then one can use a synthetic peptide immunogen to elicit antibodies to that protein. This is particularly important, since presently most amino acid sequences are generated by translation of nucleotide sequences and occasionally all one knows about a putative gene product is its deduced amino acid sequence. The protein-reactive peptide antibodies are excellent reagents for establishing the identity between a protein sequence and the protein itself. Indeed, positive, coincident, specific reactivity with antibodies elicited by two or more different peptides from an amino acid sequence may be stronger evidence that a particular protein is encoded by a given gene than a series of genetic experiments. The antibodies can also be used to determine details of the cellular location and expression of the protein. The following example illustrates such uses. Other examples and uses may be found in the recent reviews [1–3].

To survey brain-specific protein expression in the rat, Milner and Sutcliffe [4] isolated and characterized a cDNA library of brain-specific messenger RNA molecules—the thought being that since all proteins of the brain are synthesized using specific mRNA transcripts, each brain-specific protein must have a corresponding brain-specific messenger RNA. What was needed, however, was a way to link the individual cDNA clones to their encoded proteins. To do this, Sutcliffe et al [5] used the synthetic peptide immunogen approach. First, the nucleotide sequences of four of the cDNA clones were determined and the open reading frames were translated into amino acid sequence. Then using the deduced protein sequence as a blueprint, short oligopeptides were chosen and chemically synthesized. The oligopeptides were coupled to a carrier protein and used to immunize rabbits. The resulting antisera should consist of specific reagents for identifying the protein product of the corresponding open reading frame. To detect the protein product of one of the cDNA clones, p1A75, two anti-peptide sera were used to probe protein extracts of a [^{35}S]methionine-labeled pheochromocytoma cell line (PC12) that produces an mRNA species that hybridizes to the p1A75 cDNA clone. Both anti-peptide sera specifically detected a protein species of about 28,000 daltons in these immunoprecipitation studies, thereby indicating that the protein product of the p1A75 mRNA is a 28,000 dalton protein.

To determine the cellular and tissue localization of the p1A75 protein, thin, fixed sections of rat brain were reacted with the anti-peptide sera, the sections washed, and the antibody-antigen complexes visualized with horseradish peroxidase-coupled anti-rabbit immunoglobulin. The anti-peptide sera reacted specifically with the cell bodies of certain neurons located throughout the brain, including many large neurons such as the cortical pyramidal cells, thus suggesting that the 28,000 dalton protein product of the p1A75 mRNA is specifically expressed in a set of roughly homologous neurons. Furthermore, the reactivity had a granular appearance and seemed to be cytoplasmic, with the granules often concentrated in the dendritic pole of the cell as well as being frequently observed in the dendrites themselves. These observations suggest that the p1A75 protein could be involved in the synthesis or directional transport of proteins destined for dendrites or could be a component of a cytoplasmic organelle such as mitochondria.

So far we have seen how anti-peptide sera can identify the protein product of a gene and determine its cellular location, but how can one then determine the protein's biologic or enzymatic activity? If the protein has a suspected activity, then the antisera could be assayed for the ability to inhibit that activity. That is, if the anti-peptide serum can perturb an assayable protein function, then a protein containing the peptide sequence must be capable of performing that function. For

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Reprint requests to: Dr. T. M. Shinnick, Department of Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, California 92037.

Abbreviations:

HBsAg: hepatitis B virus surface antigen

example, antisera to peptides corresponding to portions of the polyoma virus middle-T protein [6] or the feline sarcoma virus *fes* gene product [7] inhibit protein kinase activity in vitro. Thus each protein must have protein kinase activity. If a function is not known, the anti-peptide antibodies can be used to purify the intact, native protein for biochemical studies. For example, the middle-T antigen of polyoma virus has been purified by immunoaffinity chromatography using anti-peptide antibodies as the immunosorbent [8]. A key advantage of the anti-peptide antibodies in this procedure is that the protein can be recovered in an enzymatically active form by gently eluting it from the immunoaffinity column by competition with an excess of peptide. Alternatively, the anti-peptide sera could be used as specific probes for the protein during various steps in a classical enzyme isolation procedure.

The second key facet of the synthetic peptide immunogen approach is that the peptide-elicited antibodies react with a small region of the protein that is chosen in advance by the investigator. As such, the anti-peptide sera are reagents for following the fate of particular portions of the protein through protein-processing pathways. For example, by using a peptide-elicited serum specific for the carboxyl-terminal 15 residues of the predicted protein of the envelope gene of Moloney murine leukemia virus, we were able to track this region from the precursor polyprotein to an intermediate product and then show that the mature protein was generated by removal of a carboxyl-terminal peptide from the intermediate [9]. Such predetermined specificity of binding can also be exploited in the analysis of DNA or RNA rearrangements that alter protein sequence, such as occurs during immunoglobulin production [10,11] or adenovirus transcription [12–14]. For example, the E1A transcription unit of adenovirus encodes three mRNAs that have overlapping nucleotide sequences and encode proteins that share amino acid sequences [12–14]. Given the common sequences, it would be very difficult to produce antibodies specific for only one of the proteins by conventional means, but with the synthetic peptide immunogen approach, the antibodies can be targeted to the sequences that differ. In this example, Feldman and Nevins [14] used a 13-residue peptide that had a sequence unique to the product of the 13S E1A mRNA to elicit antisera specific to that protein. The antisera were subsequently used as specific probes to study the function and cellular localization of this protein. In sum, the peptide immunogen approach provides a powerful set of reagents of predetermined specificity with which to investigate protein expression, processing, localization, and activity.

APPLICATIONS IN MEDICINE

The synthetic peptide immunogen approach holds promise for the development of reagents for the detection, treatment and prevention of disease. Indeed, given the exquisite predetermined specificity of the peptide-elicited protein-reactive antibodies, it may be possible to design and produce antibody reagents with precisely defined reactivities. In the detection of disease, the peptide-elicited antibodies specific for a protein of a pathogenic agent could form the basis of an immunoassay to detect or identify the pathogen. Here, the antibody specificity might be exploited to distinguish between closely related pathogens such as different serotypes of a virus (discussed below). In the treatment of disease, the protein-reactive antibodies might be useful in passive immunization protocols, antitoxin therapies, or targeted immunotherapy of neoplasia. In the prevention of disease, the peptides might serve as the basis of safe, chemically defined synthetic vaccines. Although none of these reagents has yet been sufficiently refined for use in the hospital, a brief review of our studies with hepatitis B virus will be helpful to illustrate the potential for clinical application.

Hepatitis B virus (serum hepatitis) infection leads to a fulminant hepatic disease with parenchymal cell degeneration, necrosis, and inflammation. The natural immune response to hepatitis B virus infection involves the production of neutralizing antibodies that are primarily directed against the hepatitis B virus surface antigen (HBsAg) [15–18]. This protein also carries the three serotypic markers that define the four possible serotypes of the hepatitis B virus: adw, ayw, adr, and ayr [15–18]. Hence we have chosen to study the immunology of this virus by analyzing the precise chemical nature of synthetic oligopeptides corresponding to portions of the HBsAg sequence that can mimic the serotypic markers—the thought being that if the serotypic markers are a direct reflection of the specificity of neutralizing antibodies, then we should be able to learn how to use synthetic peptides to elicit neutralizing antibodies.

Since the serotypic markers map to the HBsAg, serotype variation must be caused by a variation in the immunogenic and antigenic structure of the HBsAg, which, in turn, should be reflected in variations in the amino acid sequence of HBsAg. Therefore, we began our study by comparing the amino acid sequences of the HBsAg from three strains of hepatitis B virus in order to get an idea of which portions of the polypeptide might be responsible for the serotypic variation [19–23].

Thirteen of the 19 positions that varied between the three strains were scattered throughout the 226-residue primary sequence, but 6 changes were clustered in a 21-residue region, amino acids 114–134, thereby suggesting that this region might be involved in serotypic variation. We have synthesized and analyzed four peptides from this region [22–24]: peptides 49 and 49a, which correspond to residues 110–137 and 125–137, respectively, of the HBsAg amino acid sequence as deduced from the nucleotide sequence of a virus displaying the ayw serotype, and peptides 72 and 72a, which correspond to residues 110–137 and 125–137, respectively, of the amino acid sequence of an HBsAg of adw serotype (Table I; also [23,24]). The peptides were coupled to a carrier protein and used to immunize rabbits. The resulting antisera were assayed for the ability to react with the HBsAg of serotype adw or ayw in a solid-phase enzyme-linked immunosorbent assay (Table II). The antibodies

TABLE I. *Sequence of hepatitis B surface antigen peptides*

Peptide number	Residues	Serotype[a]	Sequence[b]
49a	125–137	y	Met-Thr-Thr-Ala-Gln-Gly-Thr-Ser-Met-Tyr-Pro-Ser-Cys
49	110–137	y	Phe-Pro-Gly-Ser-Ser-Thr-Thr-Ser-Thr-Gly-Pro-Cys-Arg-Thr Cys-Met-Thr-Thr-Ala-Gln-Gly-Thr-Ser-Met-Tyr-Pro-Ser-Cys
72a	125–137	d	Thr-Thr-Pro-Ala-Gln-Gly-Asn-Ser-Met-Phe-Pro-Ser-Cys
72	110–137	d	Ile-Pro-Gly-Ser-Thr-Thr-Thr Ser-Thr-Gly-Pro-Cys-Lys-Thr Cys-Thr-Thr-Pro-Ala-Gln-Gly-Asn-Ser-Met-Phe-Pro-Ser-Cys

[a] Serotype of HBsAg containing the peptide sequence.
[b] Underlined residues vary between the HBsAg/adw and HBsAg/ayw sequences.

TABLE II. *Serotype specificity of peptide-elicited antisera*

Peptide number	Residues	Expected serotype[a]	Antibody titer[b] against HBsAg of serotype	
			adw	ayw
49a	125–137	y	0	1280–2560
49	110–137	y	80–160	1280–2560
72a	125–137	d	1280–2560	0
72	110–137	d	640–1280	40–80

[a] Serotype of HBsAg containing the peptide sequence.
[b] Antibody titers were determined in a solid-phase enzyme-linked immunosorbent assay and are expressed as the reciprocal of the range of dilution that bound 50% of 5 pmol of antigen. A 0 indicates no activity in undiluted serum.

elicited by the peptides corresponding to residues 125–137 (49a, 72a) displayed strict serotype specificity; the anti-peptide 49a antibodies reacted only with the HBsAg/ayw and the anti-peptide 72a antibodies reacted only with the HBsAg/adw. This suggests that the region between positions 125–137 plays an important role in the expression of the two mutually exclusive serotypes d and y and that at least a portion of this serotypic difference is reflected in the four amino acid differences in this region. The antibodies elicited by the longer peptides (42, 72) bound to both HBsAg/adw and HBsAg/ayw, but showed a clear preference for the corresponding serotype. For example, the anti-peptide 49 antibodies displayed a 10- to 20-fold higher titer against HBsAg/ayw than HBsAg/adw. The slight cross-reactivity suggests that residues 110–137 carry an a determinant (the determinant or set of determinants shared in common by all hepatitis B virus strains) in addition to the y or d determinant. Indeed, as suggested, peptide 49 can be immunoprecipitated by antibodies monospecific for the a determinant(s).

From an analysis of a set of peptides corresponding to residues 110–137 that varies by one or a few amino acids we should be able to precisely define the chemical nature of the d/y serotype determinant. Obviously, such studies will provide important information on the virology and immunology of this virus, but of what possible medical significance are these peptides or anti-peptide sera? The protein-reactive antibodies could form the basis of an immunoassay for the presence of HBsAg or hepatitis B virus in serum samples, i.e., a diagnostic test for serum hepatitis. In addition, the strict serotypic specificity of the antibodies elicited by peptides 42a and 72a might be exploited to determine the d/y serotype of the virus. For example, one possible solid-phase immunoassay might involve first coating the wells of a polystyrene microtiter plate with antibodies elicited by one of the HBsAg peptides. The serum samples to be tested would then be added. The immobilized antibodies should bind any HBsAg present in the samples. After washing to remove unbound proteins, the bound HBsAg could be visualized by first adding a solution containing an enzyme-linked anti-HBsAg antibody (e.g., horseradish peroxidase-coupled goat anti-HBsAg), washing to remove unbound antibody, and then adding a chromogenic substrate for the enzyme (e.g., H_2O_2 and *O*-phenylenediamine for horseradish peroxidase). The HBsAg serotype could be determined similarly by using one of the serotype-specific antibodies as the immobilized antibody and a broadly HBsAg-specific antibody for the enzyme-linked antibody, or vice versa. Three possible advantages of anti-peptide antibodies in such assays are the ease of production of serotype-specific antibodies (currently a laborious adsorption procedure), the availability of a stable, easy-to-manufacture positive control (the peptides), and the absence of a pathogenic agent in the production of the antisera.

If the d/y serotype variation in the immune response to hepatitis B virus infection (which is defined on the basis of in vitro binding assays) is a consequence of a variation in the reactivities of neutralizing antibodies, then we have identified a region of the hepatitis B surface antigen that interacts with the biologically important neutralizing antibodies. If so, then we have identified synthetic peptide reagents that can immunologically mimic an immunogenic site of the virus that elicits neutralizing antibodies. One might expect that these synthetic peptides could form the basis of a chemically defined vaccine against hepatitis B virus. Indeed, in one preliminary experiment, the immunization of chimpanzees with peptide 49 provided at least partial immunologic protection from challenge with the homologous hepatitis B virus [23]. In addition, since there appear to be only two possible serotypes at the d/y locus, a vaccine that might provide protection against all the known serotypes of hepatitis B virus might be made by combining a synthetic peptide immunogen that elicits an anti-y response with one that elicits an anti-d response. Once again, some advantages of the synthetic peptide immunogen approach are that one does not have to grow or handle large quantities of the pathogenic agent, which is, of course, required for production of the conventional vaccine, the excellent stability of peptides at room temperature, and the ability to precisely chemically define the immunogen and thereby produce a vaccine free of any biological contamination.

DIRECTION OF FUTURE STUDIES

In basic research, the uses of peptides and the elicited antibodies have centered on identifying, localizing, and characterizing proteins and their expression. Questions amenable to the synthetic peptide immunogen approach that are yet to be fully explored include the precise chemical nature of a "tolerated" epitope, the fluidity of proteins in solution, the ability of antibodies to induce conformational changes in proteins and peptides, and the precise chemical nature of antigenic or immunogenic variation. In medicine, much effort needs to be devoted to developing and refining laboratory procedures to the degree of sophistication required for widespread clinical application. For example, a key question that must be addressed before synthetic peptide immunogens can be used as vaccines involves the use and choice of adjuvants or carriers, since the ones used in the laboratory are in general too harsh for use in humans and animals. Nonetheless, the synthetic peptide immunogen approach should generate much new basic information on protein structure, function, and immunochemistry, as well as generate novel, well-defined reagents for combatting disease.

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